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TITLE: Method of making non-pyrogenic lipopolysaccharide or A

Brief Summary Paragraph Right (26):
The genetics of lipid A biosynthesis are well described (Raetz, supra; Raetz, Ann Rev Biochem 59:129-170 (1990); and Schnaitman and Klena, supra). The majority of mutations that prevent the biosythesis of lipid A, such as mutations in lpxA, lpxB, kdsA, kdsB, kdtA, are lethal as the biosynthesis of lipid A is essental for cell survival (Rick et al, J Biol Chem, 252:4904-4912 (1977); Rick and Osborn, J Biol Chem, 252:4895-4903 (1977); Raetz et al, J Biol Chem, 260:16080-16088 (1985); Raetz, supra (1990); Raetz, supra (1993); and Schnaitman and Klena, supra). For the most part, therefore, analysis of these genes has involved the use of temperature-sensitive mutants, which only display null phenotypes under non-permissive conditions (Rick et al, supra; Rick and Osborn, supra; Raetz et al, supra; Raetz, supra (1990); Raetz, supra (1993); and Schnaitman and Klena, supra). When grown under non-permissive conditions, lpxB, kdsA, kdsB, kdtA mutants accumulate non-pyrogenic precursor forms of LPS (to about 50% of the total LPS), such as lipid X (also called 2,3-diacyl-glucosamine-1-phosphate) or lipid IVa. Conditional-mutations in kdsA and kdsB prevent the biosynthesis of 3-deoxy-D-manno-octulsonic acid (KDO) and conditional-mutations in kdtA prevent the transfer of KDO to lipid Iva (Rick et al, supra; Rick and Osborn, supra; Raetz et al, supra; Raetz, supra (1990); Raetz, supra (1993); and Schnaitman and Klena, supra). The absence of KDO moieties on lipid IVa prevents further acylation of lipid IVa resulting in the accumulation of this molecule when KDO synthesis is blocked. The necessity to add KDO to lipid IVa prior to completion of lipid A biosynthesis is further demonstrated by the fact that drugs designed to block KDO synthesis are highly toxic to gram negative bacteria Rick et al, supra; Rick and Osborn, supra; Raetz et al, supra; Raetz, supra (1990); Raetz, supra (1993); arid Schnaitman and Klena, supra). Conditional-mutations in the lpxA gene result in a 10-fold reduction of lipid A biosynthesis under non-permissive conditions by preventing transfer of .beta.-hydroxymyristate to UDP-GlcNAc, thereby preventing the synthesis of uridyldiphosphate-2,3-diacyl-glucosamine. Mutations in lpxA cause rapid cessation of growth and therefore the LpxA protein is a potential target for drug therapy. Further conditional-lethal mutants in lipid A biosynthesis also include lpxC and lpxD (Raetz, supra (1993)), which are necessary for the biosynthesis of uridyldiphosphate-2,3-diacyl-glucosamine. Recent evidence showing that ssc mutants (analogous to lpxD) of Salmonella typhimurium accumulate a pentaacyl form of lipid A indicates that this gene is also involved in lipid A biosynthesis.

Brief Summary Paragraph Right (48):
Examples of such conditional mutations that affect the biosynthesis of lipid A and result in the accumulation of non-pyrogenic LPS include, but are not restricted to, mutations in htrB, mstB, kdsA, kdsB, and kdtA (Rick et al, supra; Rick and Osborn, supra; Raetz et al, supra; Raetz, supra (1990); Raetz, supra (1993); Schnaitman and Klena, supra; Lee et al, Infect Immun, 63(3):818-324 (1995); Karow and Georgopoulos, Molec Microbiol, 5(9):2285-2292 (1991); and Karow et al J Bacteriol, 173(2):741-750 (1991)). These mutations could be introduced alone. Alternatively, any combination of mutations in the kdsA, kdsB, lpxB, kdtA, lpxC (synonym is envA), lpxD (synonyms are firA and ssc), ssc, lpxA, htrB, and the msbB genes (Rick et al, supra; Rick and Osborn, supra; Raetz et al, supra; Raetz, supra (1990); Raetz, supra (1993); and Schnaitman and Klena, supra; Lee et al, supra; Karow and Georgopoulos, supra; Karow and Georgopoulos, J Bacteriol, 174:702-710 (1992); and Karow et al, supra), which can affect the biosynthesis of lipid A and result in the synthesis of non-pyrogenic lipid

A structures could be used.

Brief Summary Paragraph Right (132):
The preparation of such non-pyrogenic bacterial vaccines is accomplished by introducing one or more mutations in the kdsA, kdsB, kdtA, lpxA, lpxB, lpxC, lpxD, ssc, pmr, htrB, and the msbB genes (Rick et al, supra; Rick and Osborn, supra; Raetz et al, supra; Raetz, supra (1990); Raetz, supra (1993); and Schnaitman and Klena, supra; Lee et al, supra; Karow and Georgopoulos, supra; and Karow et al, supra), either alone or in any combination, which affect the biosynthesis of lipid A and result in the synthesis of non-pyrogenic lipid A structures. Mutations which can be introduced into bacterial pathogens are delineated above.

CLAIMS:

5. The method according to claim 1, wherein said mutant bacterial strain comprises one or more conditional mutations selected from the group consisting of kdsA, kdsB, lpxB, kdtA, lpxC, lpxD, ssc, lpxA, htrB, and msbB.